A BRIEF ORIGINAL CONTRIBUTION

Does Prior Infection with Varicella-Zoster Virus Influence Risk of Adult Glioma?

Margaret Wrensch,1 Adriana Weinberg,2 John Wiencke,1 Helen Masters,2 Rei Miike,1 Geoffrey Barger,3 and Manon Lee1

To evaluate a possible association between varicella-zoster virus infection and glioma, the authors asked adults with glioma (n = 462) whose tumors were diagnosed between August 1, 1991, and March 31, 1994, and age-, sex-, and ethnicity-matched controls (n = 443) about their histories of chickenpox or shingles. Cases were significantly less likely than controls to report a history of either chickenpox (odds ratio = 0.4, 95% confidence interval [CI] 0.3-0.6) or shingles (odds ratio = 0.5, 95% CI 0.3-0.8). To obtain serologic support for these findings, the authors conducted double-blind enzyme-linked immunosorbent assays for immunoglobulin G antibodies to varicella-zoster virus among 167 self-reporting subjects for whom blood samples were available. Cases and controls reporting no history of chickenpox were equally likely to test positive (73% vs. 75%), but among those reporting a positive history, cases were less likely than were controls to test positive (71% vs. 85%). Despite the misclassification, an odds ratio of 0.6 was obtained using either serologic data (95% CI 0.3-1.3) or reported history of chickenpox (95% CI 0.3-1.1) in this subgroup of subjects. This suggests that adults with glioma were less likely than controls either to have had prior varicella-zoster virus infection or to have an immunoglobulin G antibody response adequate to indicate positivity. Since either explanation suggests novel mechanisms for brain tumor pathogenesis, these findings require corroboration and elaboration. Am J Epidemiol 1997;145:594-7.

chickenpox; glioma; herpesvirus 3, human

Editor's note: A companion article by Wrensch et al. appears on page 581 of this issue.

It has long been speculated that infectious agents or an immunologic response to these agents may play a role in causing, promoting, or preventing brain tumors or other cancers (1, 2). Certain viruses are known to induce glioma in test animals (1). There are intriguing but inconclusive epidemiologic data linking simian virus 40 infection with increased incidence of glioblastoma multiforme and medulloblastoma (3). Bithell et al. (4) reported a statistically significant excess of children with medulloblastoma born to mothers who had had chickenpox during pregnancy, but the results were based on only three observed cases, with 0.3 cases expected.

With regard to other infectious agents, Schuman et al. (5) showed that astrocytoma patients were significantly more likely than controls to have antibodies to Toxoplasma gondii using the Sabin-Feldman dye test, and they showed that animals exposed to Toxoplasma can develop glioma. Ryan et al. (6) could not confirm this finding in a more recent Australian study; however, they did not report histologic types for their subjects, and Schuman et al.'s findings were confined to astrocytoma.

In the ongoing San Francisco Bay Area Adult Glioma Study, subjects were asked about their histories of chickenpox and shingles. Both are caused by varicella-zoster virus, a herpesvirus which is known to have nervous system involvement (7), and we thought they might be comparatively memorable infections. Cases were significantly less likely than controls to report a history of both chickenpox (odds ratio = 0.4, 95 percent confidence interval [CI] 0.3-0.6) and shingles (odds ratio = 0.5, 95 percent CI 0.3-0.8) (8). In this report, we present serologic support for the finding...
that glioma cases were less likely than controls either to have had varicella-zoster infection or to have antibodies to this virus.

MATERIALS AND METHODS

Details on subject recruitment and interview have been given elsewhere (8). Briefly, 462 eligible adults newly diagnosed with glioma in any of six San Francisco Bay Area Counties between August 1, 1991, and March 31, 1994, participated; 443 age-, sex-, and ethnicity-matched controls were obtained through random digit dialing. Participation rates were 82 percent for cases and 63 percent for controls. In-person structured interviews asked about personal and familial medical history, demographic factors, and potential brain tumor risk factors.

Blood sample collection was undertaken partway through the study. Up to 30 ml of blood was collected from 187 cases and 169 controls in heparinized green-topped tubes. Whole blood was stored at -70°C and was transported frozen using dry ice.

Because the blood specimens were obtained primarily for polymorphism analyses, serologic studies were conducted only on a sample of available blood specimens. The subsample originally included all subjects with a negative history of chickenpox and a random sample of 43 percent of those with a positive history. Upon specimen retrieval, insufficient blood remained for serologic studies from 16 glioma cases with a negative chickenpox history. Consequently, to preserve the observed association between chickenpox history and glioma, an additional 13 glioma cases with a positive chickenpox history were deleted from serologic studies.

Serologic analysis was performed using the Varicella STAT enzyme-linked immunoassay (BioWhitaker, Walkersville, Maryland) according to the manufacturer's instructions, with modified criteria of interpretation. Briefly, test sera and controls were diluted at 1:20 and added to antigen-coated wells in a microtiter plate. Bound antibodies were revealed with peroxidase-conjugated anti-human immunoglobulin G and colorimetric substrate. Absorbances measured with a spectrophotometer were used to calculate the varicella index for each serum sample by dividing the absorbance of the test serum well by the absorbance of the manufacturer's positive control. Based on a previous study (9), a varicella index £ 0.9 indicated the absence of specific antibodies; a varicella index ≥ 1.2 indicated immunity; and a varicella index greater than 0.9 but less than 1.2 was reported as borderline, because it did not correlate with immunity nor did it exclude past infection. As performed, this test has 87 percent sensitivity and 91 percent specificity in comparison with cell-mediated immunity (9). Serologic analysis was conducted blind to both case-control status and reported history of chickenpox.

All odds ratios comparing cases with controls were adjusted for age using the SAS Logistic procedure (10).

RESULTS

History of chickenpox among all subjects and those sampled for blood and serologic analysis

Table 1 compares the odds ratios for a reported history of chickenpox among all subjects, as well as among subjects from whom a blood sample was obtained, those selected for serologic study, and those for whom adequate blood specimens remained for serologic study. Among subjects from whom blood was obtained, a significant negative association between chickenpox history and glioma persisted (table 1). As

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<tbody>
<tr>
<td><strong>Cases</strong></td>
</tr>
<tr>
<td>No. with positive history</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>All subjects</td>
</tr>
<tr>
<td>All subjects from whom blood was obtained</td>
</tr>
<tr>
<td>Subsample selected for serologic studies‡</td>
</tr>
<tr>
<td>Subsample with sufficient blood available for serologic studies‡</td>
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</tbody>
</table>

* OR, odds ratio; CI, confidence interval.
† Adjusted for individual year of age.
‡ See text for explanation of sampling strategy. These subsamples were specifically chosen to have similar odds ratios for a reported history of chickenpox as that found in all subjects from whom blood was obtained.
stated above, the subsamples selected for serologic analysis were chosen to be representative of the persons from whom blood was obtained and to preserve the observed negative association between chickenpox history and glioma.

Serologic results for immunoglobulin G antibodies to varicella-zoster virus

The odds ratio for the presence of immunoglobulin G antibodies to varicella-zoster virus for glioma cases versus controls was 0.6 (table 2). This is the same magnitude of association as that found for reported history of chickenpox, despite rather substantial misclassification (table 3); the overall sensitivity of a reported chickenpox history using immunoglobulin G as the standard was 65 percent (84/129), and the specificity was 43 percent (13/30). The proportions of cases and controls without a history of chickenpox who were antibody-positive were very similar (73 percent and 75 percent, respectively). However, 85 percent of history-positive controls but only 71 percent of history-positive cases were antibody-positive.

Prior chemotherapy and radiation did not appear to explain the lower prevalence of immunoglobulin G antibodies to varicella-zoster virus among glioma cases versus controls. Twelve percent (2/17) of antibody-negative and 27 percent (15/56) of antibody-positive glioma patients reported prior chemotherapy, while 82 percent (14/17) of antibody-negative and 89 percent (50/56) of antibody-positive patients reported radiation therapy. Two antibody-negative cases received chemotherapy 6 or 11 days before the blood drawing. Seven antibody-positive cases had chemotherapy on the day of blood drawing; the other eight had chemotherapy 4–81 days before blood collection. The average number of days between the last radiation treatment and the blood drawing was 150 for antibody-negative cases and 140 for antibody-positive cases.

### DISCUSSION

Although there was substantial misclassification between self-reported history of chickenpox and the presence of immunoglobulin G antibodies to varicella-zoster virus, the nature of the misclassification was such that the odds ratio for glioma cases versus controls for history of chickenpox was very similar to the odds ratios for positive antibodies to varicella-zoster virus. This appears to be the first time the inverse association with varicella-zoster virus antibodies has been reported; because power was limited, we choose to interpret the finding cautiously.

However, because the serologic data support the statistically significant results for reported histories of chickenpox and shingles (8), it is worth considering the implications of the finding of a negative association between glioma and varicella-zoster virus infection or antibodies to varicella-zoster virus. One possibility for an effect of this size is confounding; however, given the general ubiquity of the varicella-zoster virus, it is difficult to imagine what the confounding factors might be. Possible effects of increasing age on loss of antibody were accounted for through age adjustment with logistic analyses.

Another possibility is that having a brain tumor depresses serum immunoglobulin levels, such that it would be more difficult to detect any immunoglobulin G in cases compared with controls. On the contrary, one recent study (11) found serum immunoglobulin G levels to be significantly higher in glioma cases than in controls. The comparability of that study group to the present series is unclear; neither the origin of the control group nor the treatments received by cases were reported. The data from this current study indicate that prior radiation or chemotherapy treatments would not be likely explanations for reduced levels of immunoglobulin G among the glioma cases.

People with glioma may be less likely than controls to have a history of a wide variety of infections, but the evidence is scant. In a report by Schlehofer et al.


<table>
<thead>
<tr>
<th>Immunoglobulin G antibodies to varicella-zoster virus</th>
<th>Cases</th>
<th>Controls</th>
<th>OR* † (95% CI†)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>17</td>
<td>13</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
<td>73</td>
<td>0.6 (0.3–1.3)</td>
</tr>
<tr>
<td>Borderline</td>
<td>5</td>
<td>3</td>
<td>3.4</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>89</td>
<td>100</td>
</tr>
</tbody>
</table>

* OR, odds ratio; CI, confidence interval.
† Odds ratios for positive versus negative antibodies to varicella-zoster virus (borderline results were considered missing data), adjusted for individual year of age. If borderline results are considered positive, OR = 0.6, 95% CI 0.3–1.3; if borderline results are considered negative, OR = 0.5, 95% CI 0.3–1.1.

<table>
<thead>
<tr>
<th>Immunoglobulin G antibodies to varicella-zoster virus</th>
<th>Negative history of chickenpox</th>
<th>Positive history of chickenpox</th>
<th>All subjects</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Glioma cases</td>
<td>Controls</td>
<td>All subjects</td>
</tr>
<tr>
<td>Negative</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>72.7</td>
<td>21</td>
</tr>
<tr>
<td>Borderline</td>
<td>2</td>
<td>6.1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>100</td>
<td>28</td>
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(12), adults with newly diagnosed primary brain tumors were less likely than were population-based controls to report colds or infections during the 5 years prior to interview. The result showed a dose response, with a lower odds ratio (odds ratio = 0.3, 95 percent CI 0.1-0.8) for brain tumors appearing among those reporting three or more colds or infections per year than among those reporting 1-2 colds or infections per year (odds ratio = 0.8). The results also were very similar for men and women. Data on prior infections were not reported separately for glioma and meningioma. Schlehofer et al. interpreted this finding as indicating that general activation of the immune system might play a role in influencing these tumors. The implication seems to be that cancer cells, in general, might be more readily destroyed with a heightened immune system. In partial support of this contention, Abel et al. (13) reported a decreased history of prior colds and several childhood infections, including chickenpox, among newly diagnosed cancer patients (stomach, colorectal, breast, and ovarian carcinoma) compared with controls. They thoroughly discussed the previous literature on infections and cancer risk. Hypotheses have included either that infections decrease the chance of cancer development or kill existing cancer cells; alternatively, infections may be less likely to arise in individuals who are more susceptible to cancer or who are developing cancer. Reporting bias by cases, diminishing the reported severity of their prior illnesses, was also suggested. However, this bias could not explain the present serologic findings. Another, more speculative explanation is that if a virus or viruses with some cross-reactivity to varicella-zoster virus influence glioma development, individuals with stronger immunity to varicella-zoster virus might be less susceptible to glioma.

Clearly, the role of viral and other infections in brain tumor etiology requires further epidemiologic investigation. This is especially important given the extremely poor prognosis of most brain cancers and the paucity of available knowledge with which to develop meaningful preventive strategies. Our results suggest that self-reported history of chickenpox is an unreliable indicator of serologic positivity. In addition to the need for replication of these results, it would be valuable to compare glioma cases and controls for antibodies to herpes simplex virus, cytomegalovirus, and Epstein-Barr virus. Herpes simplex virus is another herpesvirus that establishes latency in the nervous system, while cytomegalovirus and Epstein-Barr virus are herpesviruses that infect the central nervous system without establishing latency.

ACKNOWLEDGMENTS

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REFERENCES